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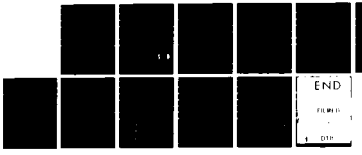

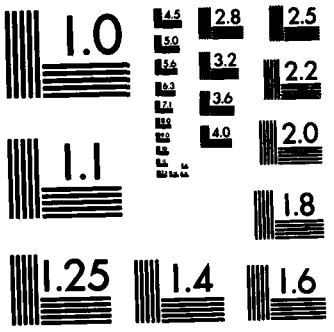

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complete. The average affinity constant of the antibody (k_m) was 1.75×10^{10} liters/mol. The sensitivity was 1 ng per assay or 10 ng/ml. Among the other trichothecenes tested only H T-2 cross-reacted significantly (10.3%).

Detection of T-2 Toxin by an Improved Radioimmunoassay

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The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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ABSTRACT

T-2 toxin in serum, urine and saline was analyzed by a modified radioimmunoassay procedure. The specimens were added directly to the assay tubes without extraction steps. The reaction between antibody and ligands was optimized at one hour. Albumin-coated charcoal was used to separate bound from free radioactivity. Quenching which occurred with hemolyzed specimens was corrected with a wet oxidation process using 60% perchloric acid and 30% hydrogen peroxide. The shorter incubation times resulted in an assay that takes less than 6 h to complete. The average affinity constant of the antibody (k_m) was 1.75×10^{10} liters/mol. The sensitivity was 1 ng per assay or 10 ng/ml. Among the other trichothecenes tested only H T-2 cross-reacted significantly (10.3%).

The occurrence of mycotoxins in nature depends on such factors as temperature, humidity, food processing methods and type of food product. Almost all food products can be contaminated with fungi and carry the potential for animal and human intoxication. The ingestion of food products contaminated with toxins from certain fungi such as Fusarium and Trichoderma has been associated with toxic manifestations (1-3). Epidemics of alimentary toxic aleukia attributed to trichothecene ingestion, specifically T-2, have been reported in certain regions of the USSR (4). Due to its potential for mass intoxication, early and rapid methods for the detection of T-2 toxin in the environment, in food products and biologic samples such as serum or urine are essential. The methods that are presently available, such as bioassays, high-performance liquid chromatography and gas chromatography, are not readily available to smaller laboratories due to the need for sophisticated instrumentation and skilled personnel. A radioimmunoassay procedure reported by Chu et al. has simplified the testing process (5). We have modified this assay and further characterized the antibody.

MATERIALS AND METHODS

Chemicals. T-2 toxin standards and other trichothecene mycotoxins were purchased from Calbiochem-Behringer Corporation, La Jolla, Cal., and Mycolabs Company, Chesterfield, Mo. Tritiated T-2 with a specific activity of 13.5 Ci/mmol was prepared according to the procedure of Wallace et al. (5). Rabbit anti-T-2 hemisuccinate antibody was produced by multiple site injections of T-2 hemisuccinate-bovine serum albumin (HS-BSA) as described by Chu et al. (6). Known concentrations of T2 toxin added to pooled human serum and urine were used as biologic samples. Albumin-coated charcoal was prepared by adding 1.25 ml of 22% bovine albumin (Sigma Chemicals Corporation, St. Louis, Mo.) to 1.25 g neutral Norit A Charcoal (Sigma). Distilled water was added to a final volume of 50 ml. The mixture was stirred constantly while being added to the tubes. Radioactivity was counted in a Searle 6880 liquid scintillation counter.

Antibody titration and determination of affinity. The optimal titer of the rabbit anti-T-2 antibody was determined by obtaining the dilution of the antibody that resulted in the binding of 50% of the radioactivity in a trace amount of radiolabeled ligand as described previously (6). This dilution was used in all the subsequent experiments. The average affinity constant was determined by incubating increasing amounts of radiolabeled T-2 and increasing mixtures of unlabeled T-2 and a constant amount of the antibody according to the method of Odell et al. (7).

T-2 radioimmunoassay. One hundred µl of the T-2 standards or the unknown samples in 0.1 M sodium phosphate buffer were added to 10 x 75 mm borosilicate tubes in triplicate. Fifty µl of the ^{3}H-T-2 toxin (about 20,000 dpm) and 50 µl of the working dilution of the rabbit anti-T-2 antibody were then added. After 1 h incubation at 4^{o}C, 200 µl of the albumin-coated charcoal were pipetted. The tubes were immediately mixed and centrifuged at 2,000 x g for 30 minutes. The supernatants were decanted into scintillation vials and the radioactivity was counted. For hemolyzed samples, 200 µl of

60% perchloric acid and 400 µl of 30% hydrogen peroxide were added sequentially. The vials were incubated at 37°C for 30 minutes then counted in a refrigerated liquid scintillation counter.

RESULTS

A 1:10 dilution of the antiserum resulted in binding 50% of the trace amount of ^{3}H-T-2 (Fig. 1). The average affinity constant (k) was 1.75×10^{10} liters/mol calculated from the free antigen concentration at 50% saturation (Fig. 2). Incubating the reactants for more than one hour did not result in increased binding (Fig. 3). When the unextracted samples were tested, the recoveries ranged from 59.8 to 126.8% with a mean recovery of 93.6 $\pm$ 16.9% ($\pm$ SD). At higher concentrations, T-2 added to biologic samples inhibited the assay and was brought to detection range which was between 1 and 10 ng (Fig. 4) by serial dilution. The interassay coefficient of variation was 9%.

In the cross-reactivity experiments, 2.6 ng of T-2 were needed to inhibit the reaction by 50%, while H-T-2, T-2 triol, and diacetoxyscirpenol (DAS) required 25.1, 2490 and 2510 ng, respectively, to produce the same degree of inhibition (Fig. 5). The relative potency of the cross-reacting trichothecenes calculated as a percentage of the T-2 standards at 50% inhibition was 10.3% for H T-2 and 0.1% for T-2 triol and DAS. Of the other trichothecenes studied, T-2 Tetraol, Verrucarol and Vomitoxin showed less than 20% inhibition at 10 µg. Verrucarin exhibited less than 5% inhibition at 10 µg and Roridin did not inhibit the reaction.

DISCUSSION

Detection methods, which are rapid, simple and sensitive, are needed for the determination of mycotoxin contamination and in biologic specimens for the assessment of levels of toxin concentration. The ideal assay would be one that is both sensitive and highly specific. Physicochemical methods, that have been developed, are not widely used because of the need for skilled personnel and sophisticated instrumentation.

The radioimmunoassay for T-2 toxin, although specific for one particular toxin, measures a highly toxic and readily produced member of the trichothecene group (8). We have modified this assay resulting in a total time of less than 6 h. Extraction steps used in the previous assays were omitted. Blood and urine samples with T-2 toxin added were used directly in the assay tubes. The sample requirement was minimal, only 100 µl per tube. The sensitivity was comparable to the previously reported assay (5), 1 ng per assay or 10 ng/ml.

A Scatchard plot of our experiment gave a biphasic curve, an indication that more than one antibody species was present. We ran a saturation curve for the antibody and determined the average affinity constant according to the method of Odell et al. (7) from the concentration of free antigen at 50% antibody saturation. Although the average affinity constant was high, ($k_m = 1.75 \times 10^{10}$ liters/mol), the optimal titer used in our assay was low (1:10). This is however, consistent since the titer also reflects on the total specific antibody concentration. Useful radioassays have been developed with low titer antibodies as long as the affinities are adequate (10^9 liters/mol or greater) (9).

During the initial experiments, hemolyzed blood produced quenching which resulted in the overestimation of T-2 in these samples. We have used a wet oxidation method as described by Mahin, Lofberg (10) to correct this problem. The hemolyzed samples we have analyzed, which were stored for weeks, cleared completely. Although precipitation occurred in some tubes, this did not contribute to any counting problems. The samples can also be oxidized prior to incubation with the antibody, although we preferred the post-incubation oxidation process. This method of oxidation has also been recommended for tissue samples of 100 mg or less.

H T-2 cross-reacts with T-2 significantly (10.3%),therefore caution must be exercised in reporting concentrations in the presence of H T-2. The other trichothecenes examined did not cross-react to any significant degree. The narrow range of detection makes it necessary to dilute specimens serially to bring samples at high concentration to

within the sensitive range of the assay. We routinely dilute samples that are greater than 10 ng/ml in order to make use of the sensitive portion of the standard curve. Aside from problems common to other isotopic assays such as isotope disposal, the significant cross-reaction with H T-2 can make quantitation difficult. The presence of T-2 triol and DAS in very high concentrations, as may occur in multiple massive toxin exposure can potentially create problems in the interpretation of results. The newer antibody production techniques, may offer a solution to the low antibody titer; efforts are being made in other laboratories to produce monoclonal antibodies to T-2 toxins.

The assay in operation for several months now, has been most useful in measuring T-2 concentrations in animal toxicologic studies and evaluating detoxification protocols in experiments where only T-2 toxin was used. The assay reproducibility and sensitivity is adequate for most laboratory needs. The omission of the extraction steps, shorter incubation times and modification of the separation process has resulted in a more rapid and simple assay.

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FIGURE LEGENDS

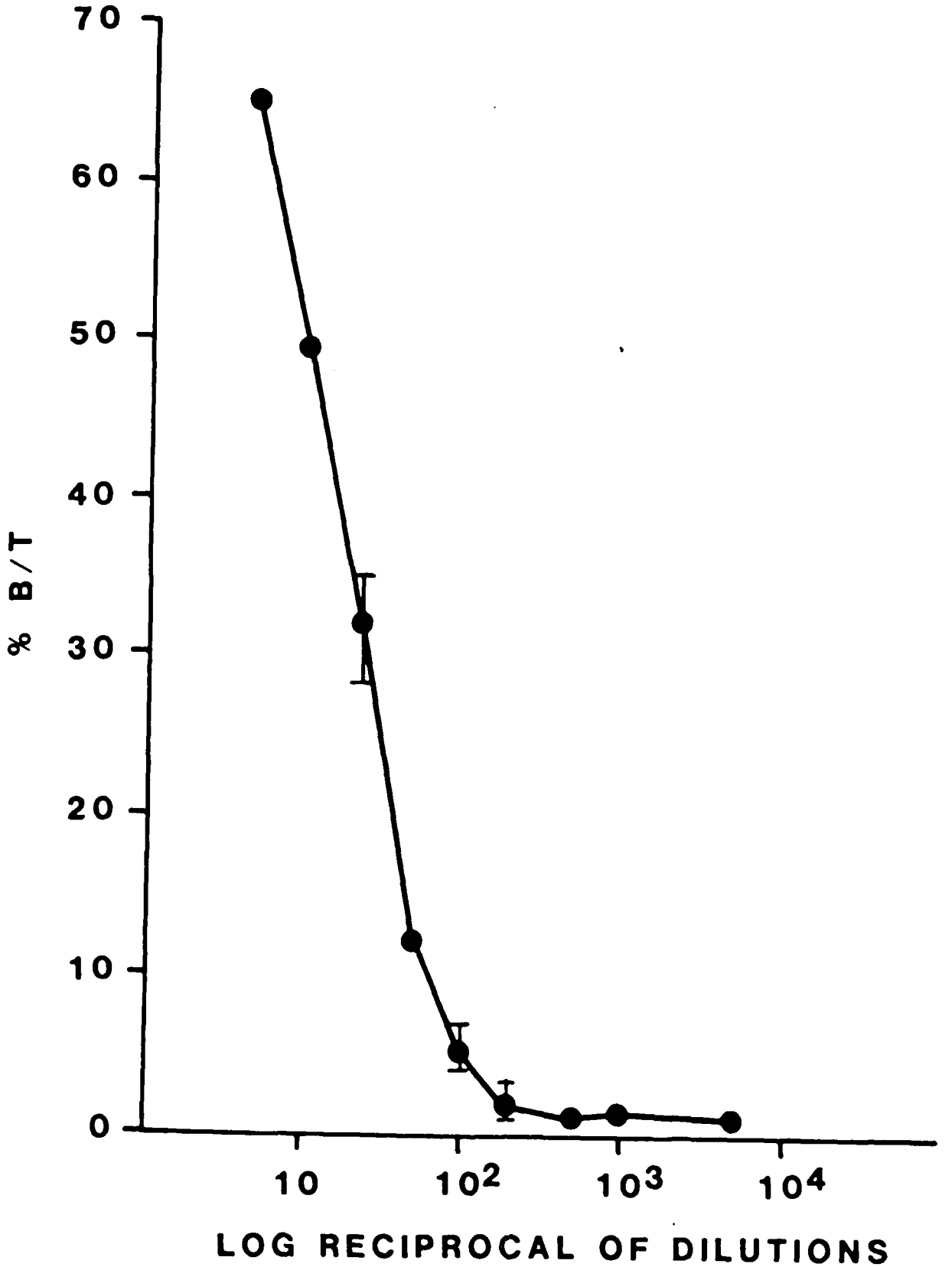
FIG. 1. Primary titration of rabbit anti-T-2 antibody to determine the dilution that will bind 50% of the radioactive ligand (Mean + 2 SD are shown).

FIG. 2. Saturation curve for antibody against T-2 toxin to determine the average affinity constant. Concentration of antigen at 50% saturation is 1.75×10^{10} = liters/mol

FIG. 3. Determination of optimal incubation time for the reaction of anti-T-2 antibody with ^{3}H T-2 and unlabeled T-2.

FIG. 4. Typical standard inhibition curve. Sensitivity is 1 ng; two standard deviations greater than the uninhibited tubes (Mean + 2 SD are shown).

FIG. 5. Cross-reactivity between T-2 and other trichothecene mycotoxins for the binding sites of antibody; 2.6 ng of T-2 inhibited the reaction by 50%, whereas, 25.1, 2,490 and 2,510 ng of H T-2, T-2 Triol and DAS were required to produce the same effect (Mean + 2 SD are shown).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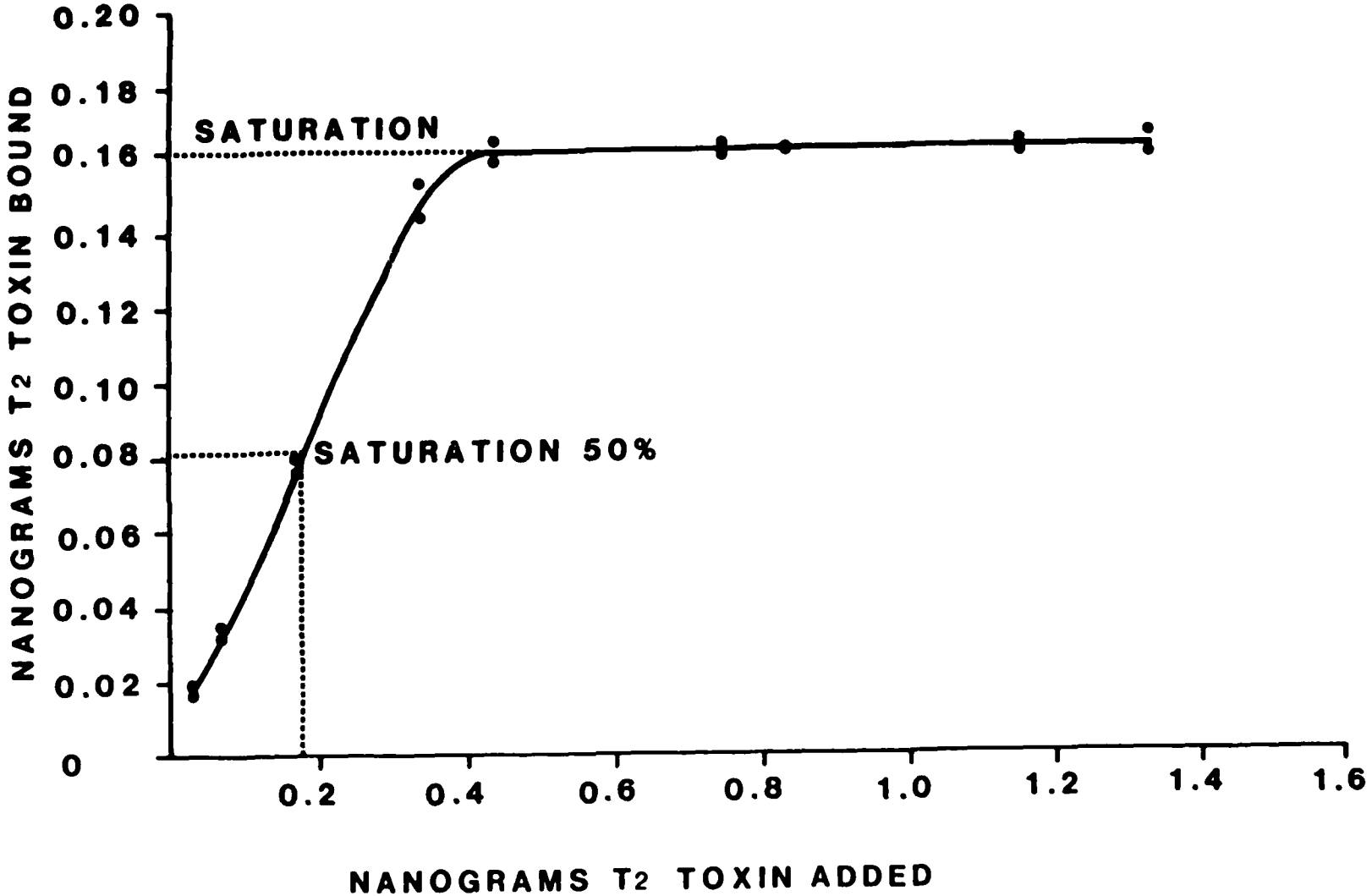
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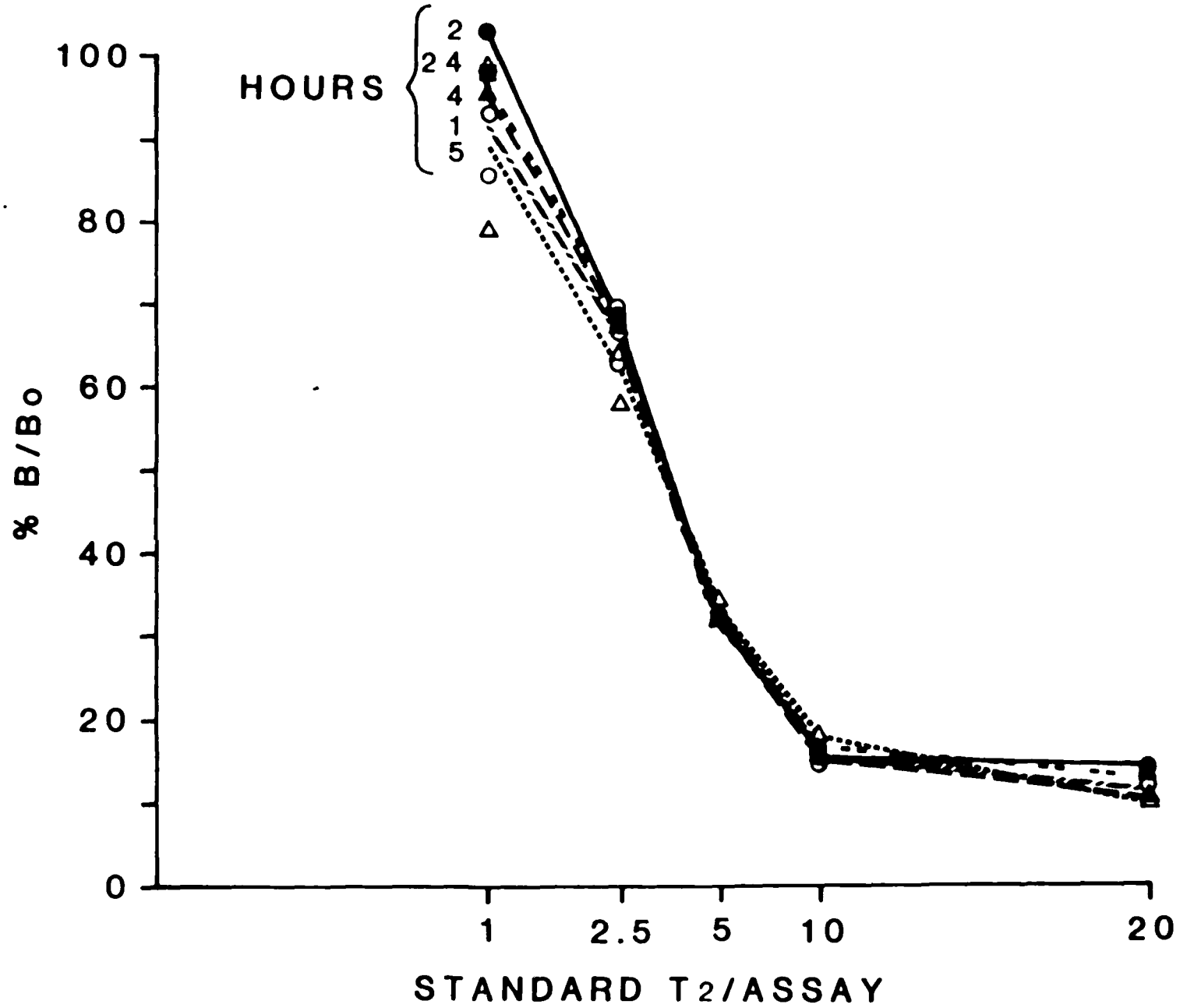
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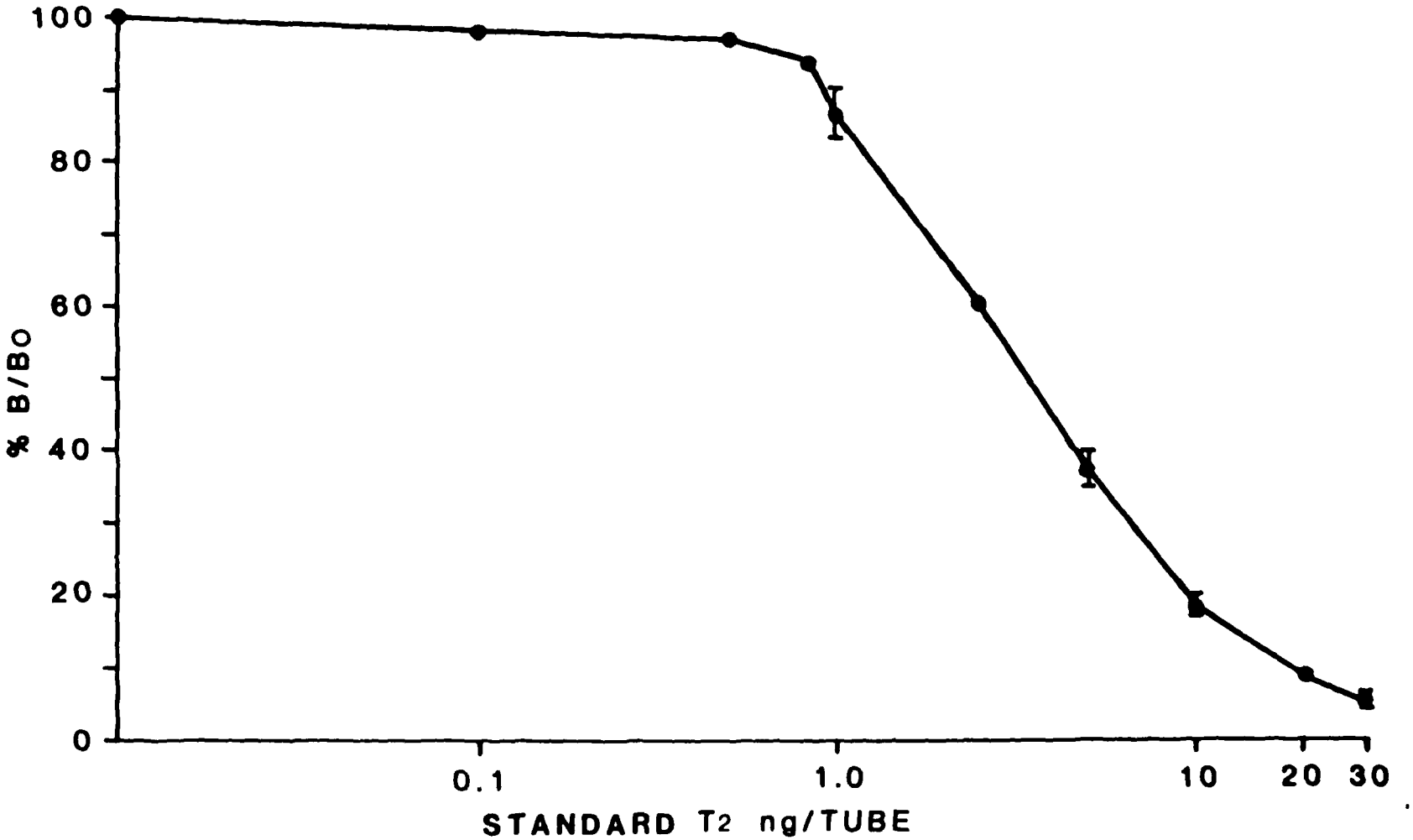
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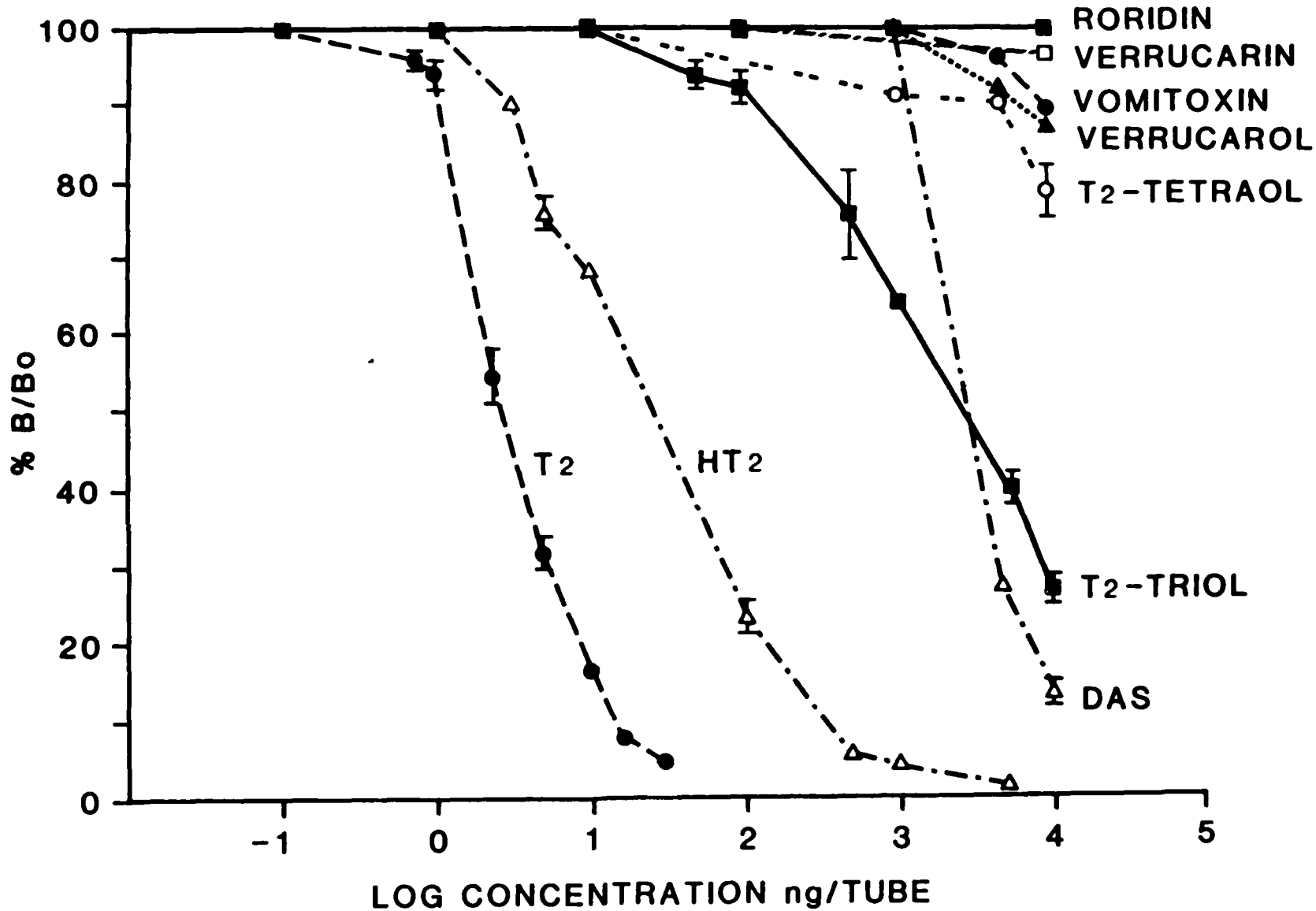
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